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N-β-Alanyldopamine Metabolism for Puparial Tanning in Wild-Type and Mutant *Niger* Strains of the Mediterranean Fruit Fly, *Ceratitis capitata*

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N-β-Alanyldopamine (NBAD) metabolism was investigated in wild-type and melanic mutant *niger* strains of the Mediterranean fruit fly, *Ceratitis capitata*, during the process of pupariation. When radioactive β-alanine (BALA) was injected at the onset of pupariation, the wild-type puparium incorporated 70% of the label, whereas less than 5% was detected in the *niger* puparium. BALA was catabolized and eliminated as carbon dioxide substantially more in the mutant than the wild-type strain. Significant differences between the two strains were found in the composition of catecholamines extracted from fully sclerotized puparial cuticle. NBAD levels in *niger* were five times lower than those in the wild type, whereas dopamine (DA) levels were 20 times higher. The concentration of NBAD in hemolymph also was greatly reduced in *niger*, whereas DA and *N*-acetyldopamine levels remained high during pupariation. The synthesis of NBAD was assayed both *in vivo* and *in vitro*, revealing that the conversion of BALA into NBAD was several fold higher in the wild-type than in the *niger* strain. Because NBAD hydrolase activity was found to be similar in both strains, an impairment in NBAD synthesis appears to be responsible for the melanic phenotype. We also found that NBAD synthesis in the wild type was restricted to a narrow developmental window, which overlaps the time period in which puparial tanning occurs. Because we were able to induce *niger*-wild-type cuticle mosaics in heterozygous embryos, NBAD synthetase appears to be expressed by the epidermis. We conclude that the *niger* mutant is defective in NBAD synthesis and that this defect prevents normal pigmentation. Published by Elsevier Science Ltd

Catecholamines Cuticle Hemolymph Pigmentation Sclerotization Puparium Dopamine *N*-Acetyldopamine β-Alanine

INTRODUCTION

Sclerotization of insect cuticle results in hardening, stiffening and dehydration of the exoskeleton primarily by formation of cross-links and other bonds between nucleophilic groups of cuticular proteins, chitin, and *N*-acetyldopamine quinones (Andersen, 1979, 1985; Schaefer *et al.*, 1987; Sugumaran, 1988; Hopkins and Kramer, 1992). *N*-Acetyldopamine (NADA) and *N*-β-alanyldopamine (NBAD) play key roles as precursors of several quinoid cross-linking agents involved in the sclerotization of insect cuticles (Karlson and Sekeris, 1962, Hopkins *et al.*, 1982). The final step in the biosynthesis of these catecholamines is the acylation of dopamine with either acetate or β-alanine (BALA) (Brunet, 1980; Kramer and Hopkins, 1987; Wright, 1987; Hopkins and Kramer, 1992). Although some information about the enzyme(s) catalyzing the synthesis of NADA has been reported (Maranda and Hodgetts, 1977; Shampentong *et al.*, 1987), relatively little is known about the enzyme(s) catalysing the synthesis of NBAD (Wright, 1987; Krueger *et al.*, 1989, 1990).

At the time of cuticle sclerotization, the melanic

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mutants, *black* and *ebony*, of *Drosophila melanogaster* (Jacobs, 1985, Wright, 1987); and the *black* mutants of the red flour beetle, *Tribolium castaneum*; the German cockroach, *Blattella germanica*; and the Mediterranean fruit fly, *Ceratitis capitata* (Kramer *et al.*, 1984; Roseland *et al.*, 1987; Czapla *et al.*, 1989; Wappner *et al.*, 1996) contain abnormally low amounts of NBAD but high levels of dopamine in whole-body or cuticle extracts. Except for *ebony*, the normal phenotypes of these mutants can be rescued by injection of BALA, suggesting a deficiency of NBAD synthesis because of low amounts of BALA available for N-acylation of dopamine. However, like *ebony* of *D. melanogaster*, *niger*, another melanic mutant of *C. capitata*, could not be prevented from melanizing the puparium by BALA injections, suggesting a defect in the NBAD synthetase system (Wappner *et al.*, 1996).

Recently, we found that NBAD is the primary cuticle sclerotizing agent during the process of pupariation of the Mediterranean fruit fly, *C. capitata* (Wappner *et al.*, 1995). Whole-body extracts of a melanic mutant, *Black pupa*, showed very low levels of NBAD, whereas the dopamine concentration was at least 10-fold higher than that in the wild type. *Black pupa* was found to have reduced levels of BALA as well and, after an exogenous supply of this amino acid was administered, both the wild-type phenotype and the normal catecholamine profile were recovered (Wappner *et al.*, 1996).

In this study, we further investigated the biochemical basis for melanization in *niger*, and the synthesis and hydrolysis of NBAD as a possible cause of puparial melanization.

MATERIALS AND METHODS

Insect rearing and synchronization of pupariation

C. capitata strains were obtained from the Instituto de Genética INTA, Castelar, Argentina. The reference strain in all the experiments was the wild-type ARG-17. The *niger* mutant strain used in this paper (nig^1 alelle) was generated from ARG-17 flies by treatment with ethyl methane sulphonate (EMS) (Manso and Lifschitz, 1979). Medfly larvae were reared in a carrot-yeast-corn medium and adults were fed a mixture of yeast and sugar as previously described (Quesada-Allué *et al.*, 1994). The insects were maintained at 23°C, 55–80% RH using a photoperiodic regime of L16 h: D8 h as described in Quesada-Allué *et al.* (1994).

To synchronize the cultures, the 'zero time' of metamorphosis (onset of pupariation) was as defined by Rabossi *et al.* (1992). At this stage, the larvae remain completely immobile and did not respond to mechanical stimuli.

Chemicals

Dopamine (DA), NADA, DOPA (3,4-dihydroxyphenylalanine), α-methyl-DOPA, and BALA were from Sigma Chem. Co. ($P^{14}C$)-BALA (54.5 mCi/mmol) was from New England Nuclear. NBAD was synthesized as described in Yamasaki *et al.* (1990).

Injection of β-alanine

Zero time prepupae were microinjected ventrally through the last intersegmental grove with 0.5 μL [^{14}C]-BALA dissolved in insect Ringer's solution (Chen, 1968) at room temperature (Rabossi *et al.*, 1992). A 5 μL Hamilton syringe connected through a Tygon cannula to a 90 pm stainless steel needle was used routinely.

Distribution of radioactivity in [^{14}C]-BALA-injected prepupae

Immediately after the injection, each prepupa was placed in a tightly closed 25 mL flask containing a strip of Whatman 3MM paper saturated with 1 M KOH for collection of CO_2 and a small vial containing 0.5 mL of distilled water. After 24 h, the paper strips were dried under an infrared lamp. The corresponding prepupae were dissected immediately on rectangular pieces of Whatman 3MM paper, and the body contents were spread out on the paper with a small spatula and dried as above. Puparial cuticles were washed with distilled water, blotted on paper, and dried. The puparia and corresponding papers were introduced separately into vials containing scintillation fluid for radioactivity determination in a liquid scintillation counter. Each determination was done in duplicate using 10 prepupae.

Catecholamine analysis

Catecholamines were extracted from either cuticle or hemolymph according to Morgan *et al.* (1987) with slight modifications. Hemolymph (2–6 μL) was extracted (Wappner *et al.*, 1995) and homogenized in 500 μL of 1.2 M HCl plus 5 mM ascorbic acid in ground glass tissue grinders. The homogenates then were centrifuged at 14 000 rpm for 10 min and lipids were extracted from the supernatant by addition of 500 μL of chloroform followed by vigorous shaking and centrifugation. The upper phase was then separated, incubated for 10 min at 100°C, adsorbed onto alumina equilibrated with 0.5 M Tris–HCl buffer pH 8.6, and desorbed with 1 M acetic acid. The samples were analyzed by reversed phase liquid chromatography (LC) using a C-18 column and electrochemical detection. Two different mobile phases were used: mobile phase I was 1 mM sodium octyl sulfate, 2.5 mM KCl, 1 mM Na_2EDTA, 0.1 M phosphoric acid, and 6% acetonitrile adjusted to pH 2.2; mobile phase II was 0.09 mM sodium octyl sulfate, 0.1 mM EDTA, 0.1 M phosphoric acid, and 10% methanol adjusted to pH 3.0. α-Methyl DOPA was used as an internal standard to calculate the efficiency of catecholamine recovery. For the preparation of puparial extracts, six 48-h pupae (fully sclerotized) were dissected under distilled water saturated in phenylthiourea (PTU), and the puparial cuticles cleaned by scraping the internal surface with a bent needle to remove the remaining tissues. Then the cuticles were

blotted onto filter paper, rinsed several times with water saturated with PTU, blotted again, and weighed. Puparia were homogenized in 1.2 M HCl containing 5 mM ascorbic acid. After centrifugation, the catecholamines in the supernatants were subjected to alumina recovery and analyzed by LC as described above.

Determination of NBAD synthetase activity

To determine NBAD synthetase activity *in vivo*, six prepupae from each strain were injected at the onset of pupariation with [^{14}C]-BALA and kept for 3 h at 23°C. The prepupae then were homogenized in 400 μL of 50 mM Tris–HCl pH 7.6. After centrifugation, the soluble material was delipidated with one volume of chloroform, and the proteins were precipitated with 20 μL of 6.6 M perchloric acid. The supernatants were analyzed by LC as described above, and fractions (0.5 mL) were collected in vials containing scintillation fluid and counted. α-Methyl-DOPA was used as an internal standard to determine the efficiency of the extraction.

To measure NBAD synthetase activity *in vitro*, 2 h prepupae were homogenized in 400 μL of 100 mM sodium borate (pH 8.0) saturated with phenylthiourea (PTU) containing 10 mM $MgCl_2$, 10% glycerol, and a mixture of protease inhibitors (100 μg/ml phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 1 μg/ml pepstatin and 1 μg/ml E-64). The homogenates were centrifuged and the supernatants used as enzyme sources. The reaction mixture contained 10 mM dopamine, 1 mM ATP, 5 mM $MgCl_2$, and 100 000 cpm of [^{14}C]-BALA in 50 mM sodium borate (pH 8.4). The samples were incubated for 15 min at 22°C, and the reaction was stopped by the addition of perchloric acid to a 0.33 M final concentration. Catecholamines in the extracts were resolved by LC as described above. Radioactivity in the fractions (0.5 mL) was measured in a liquid scintillation counter. A mixture of catecholamine standards was separated before and after each analysis. Detection was by recording absorption at 280 nm. The retention time of BALA was assessed using a radiolabeled standard. Protein concentration in the homogenates was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard protein.

Determination of NBAD hydrolase activity

[^{14}C]-NBAD, enzymatically synthesized and purified with alumina, was used as the substrate. After recovery from alumina, LC analysis showed that the only radiolabeled substance was NBAD. Two h prepupae were homogenized with 50 mM phosphate pH 7.2 containing 50 mM EDTA, 10% glycerol, and saturated with PTU. To measure NBAD hydrolyase activity, extracts of *niger* and wild-type strains containing equal amounts of protein were incubated at 30°C for 30 min with radiolabeled NBAD in the homogenization buffer without glycerol. The reaction was stopped with perchloric acid, and the amount of radiolabeled NBAD was determined as above.

Induction of niger*/wild-type genetic mosaics*

The *niger* and wild-type strains were crossed reciprocally, and 1 week later, eggs were collected for 2 h and immediately X-irradiated for 5–6 s at 12.5 rads/s. 48 hours after the onset of pupariation, the puparium phenotype was recorded.

RESULTS

Utilization of BALA in niger *and wild-type strains*

NBAD levels in *niger* whole body extracts were found to be lower than those in extracts of the wild type, whereas DA and BALA levels were higher (Wappner *et al.*, 1996). Consistently, upon injection with BALA, the *niger* mutant failed to recover either the normal phenotype or the normal catecholamine composition. In order to understand the biochemical defect responsible for the *niger* mutant, the metabolism and distribution of BALA and NBAD were investigated.

When [^{14}C]-BALA was injected as a tracer at the onset of pupariation, the incorporation of radioactivity into the homozygous *niger* puparium was very low when compared with the wild type (Fig. 1). This indicated that BALA was not utilized highly for cuticle sclerotization in the mutant. In *niger*, the bulk of the [^{14}C]BALA was metabolized and eliminated as [^{14}C]CO_2 via respiration. The residual radioactivity present in the body contents was similar in both strains. Interestingly, for heterozygotes, the incorporation of radioactivity into the puparium (62.4 ± 1.7%) was similar to that for the wild type. This result is consistent with previously reported genetic data, because this mutation is phenotypically recessive (Manso and Lifschitz, 1979).

Catecholamine levels in cuticle and hemolymph during development

Acidic extracts were analyzed to determine the catecholamine composition in cuticle and hemolymph at different times after the onset of pupariation. The dopamine

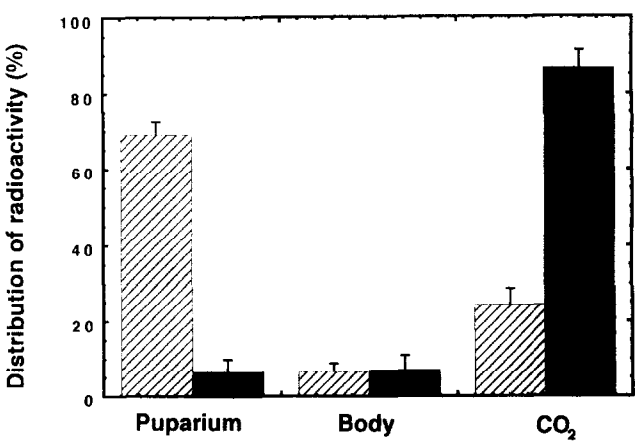

FIGURE 1. BALA utilization by wild-type and *niger* strains of *C. capitata*. Radioactivity was measured in each of the fractions 24 h after injecting [^{14}C]BALA. Hatched bars: wild type; solid bars: *niger* mutant. Means ± SEM (n = 10).

content of the puparium was 20 times higher in *niger* than in the wild type (Fig. 2). However, the levels of NBAD in the latter were 5-fold higher than those of *niger*. NADA and *N*-β-alanylnorepinephrine (NBANE) were extracted in trace amounts from the puparia of both strains.

NBAD titers were much lower in *niger* hemolymph than in the wild-type strain, whereas dopamine content was always much higher in the mutant (Fig. 3A and B). The concentration of NADA in *niger* hemolymph was remarkably high 10 h after the onset of pupariation, but low in the wild-type (Fig. 3C). In addition to high concentrations of dopamine, the LC chromatograms showed rather substantial amounts of DOPA in whole-body extracts of *niger* (approximately 50 pmoles/mg of wet weight), but little if any in the wild-type strain (less than the detection limit of 0.1 pmole/mg).

Synthesis and hydrolysis of NBAD

The above data suggest that *niger* mutants are defective in their ability to synthesize NBAD. To determine whether this hypothesis is true, NBAD synthesis in *niger* and wild-type strains was investigated *in vivo* and *in vitro* using [^{14}C]BALA as a substrate. When BALA was injected at the onset of pupariation, two radiolabeled peaks were detected in the LC chromatogram of the wild-type extract, but only one major peak in that of the *niger* extract (Fig. 4A). The retention times of the two peaks in two different mobile phases were coincident with those of BALA and NBAD. Other β-alanylated catecholamines, such as NBANE, were not synthesized in detectable amounts. In agreement with the dosage experiments (Figs 2–3), BALA conversion into NBAD was substantially greater in the wild-type strain than in the *niger* mutant (Fig. 4A).

In cell-free *in vitro* experiments, the presence of millimolar concentrations of unlabeled NBAD did not inhibit the coupling reaction (data not shown) and, therefore, nonradioactive 2 mM NBAD was included in the incubations to dilute the radioactive product that is subject to degradation by a hydrolysing enzyme (see below). The results indicated that the conversion of BALA into NBAD was about four times higher in the wild-type than in the *niger* strain (Fig. 4B). This suggests that NBAD synthetase is defective in the *niger* mutant, which would explain the mutant's inability to utilize BALA for NBAD synthesis and subsequent sclerotization reactions.

The possibility that overexpression of a NBAD hydrolyzing enzyme accounts for the *niger* phenotype as well as for the above results was discounted, because we determined that NBAD hydrolase activity in *niger* was

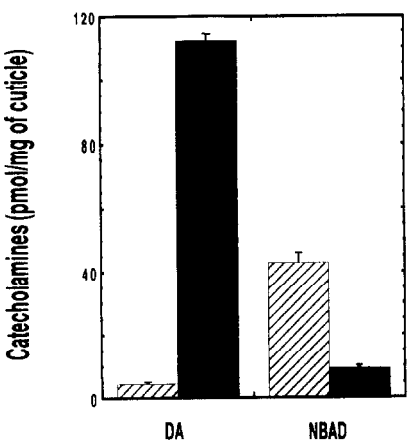

FIGURE 2. Catecholamine composition of fully sclerotized puparia of *C. capitata* extracted 48 h after the onset of pupariation. Hatched bars: wild type; solid bars: *niger* mutant. DA, dopamine; NBAD, N-β-alanyldopamine. Concentrations were calculated by comparing the retention times of cuticle and standard catecholamines resolved by LC. α-Methyl DOPA was used as the internal standard to determine the recovery of catecholamines from alumina (see Materials and Methods section). Means ± 0.5 range (n = 2).

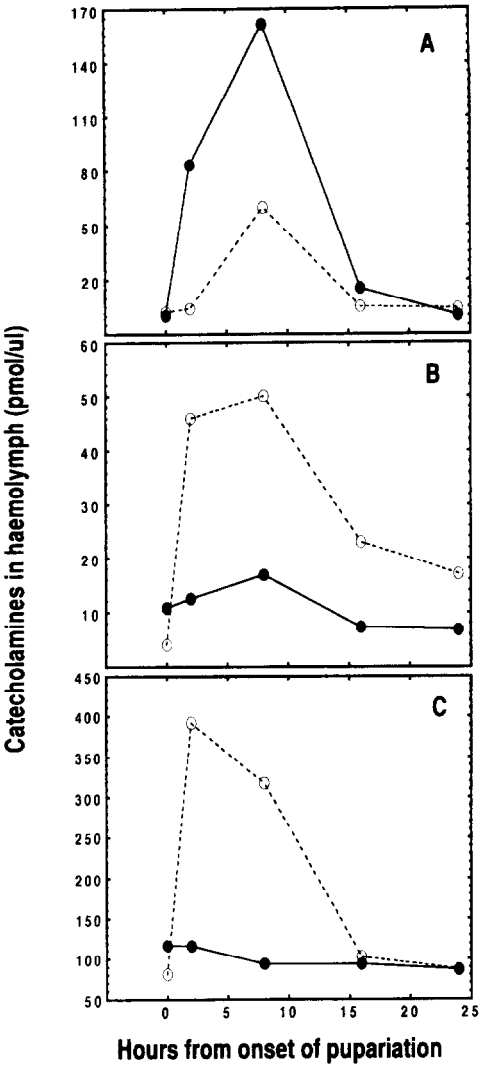

FIGURE 3. Time course of catecholamine concentrations in hemolymph during pupariation of *C. capitata*. Zero time is the onset of pupariation. Open circles, wild type; solid circles, *niger* mutant. Panel A, N-β-alanyldopamine; B, dopamine; C, *N*-acetyldopamine. Concentrations were determined by comparing hemolymph and standard catecholamine peak areas resolved by LC. α-Methyl DOPA was used as the internal standard to assess the recovery of catecholamines from alumina (see Materials and Methods section). Mean values of two determinations.

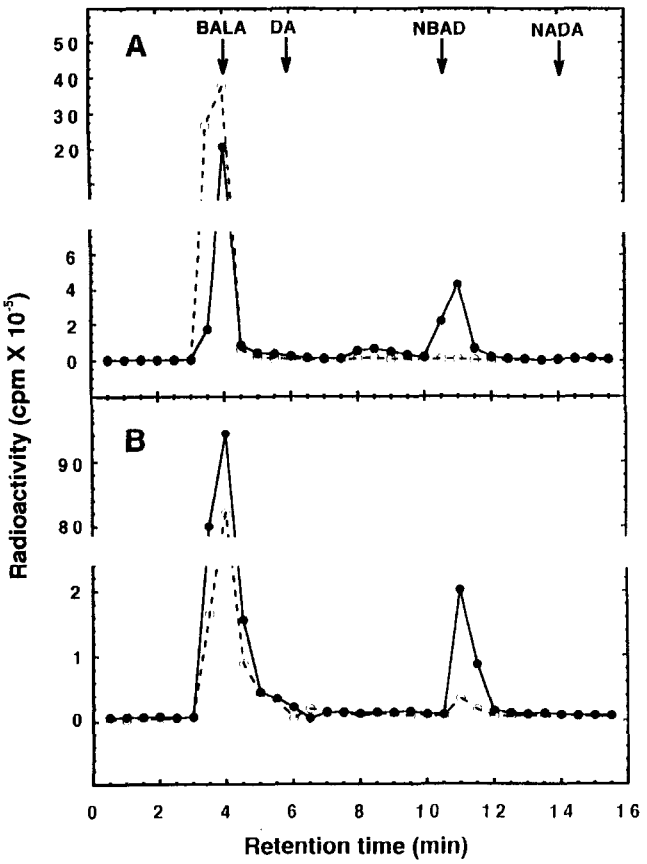

FIGURE 4. *N*-β-Alanyldopamine synthetase activity in prepupae of *C. capitata*. Panel A, LC profile of radiolabeled substances after injection with [^{14}C]BALA. Panel B, NBAD synthesis *in vitro*. In both cases, mobile phase 2 (see Materials and Methods section) was used. Solid line, wild type; dashed line, *niger* mutant. Arrows indicate the retention times of standard compounds resolved using the same chromatographic conditions. β-Alanine, BALA; DA, dopamine; NBAD, *N*-β-alanyldopamine; NADA, *N*-acetyldopamine. α-Methyl DOPA was used as the internal standard to calculate the efficiency of the extraction in the experiment performed *in vivo*. Counts per minute (cpm) are per 1 mg protein assayed. Data are means of two or more experiments with each of the mobile phases.

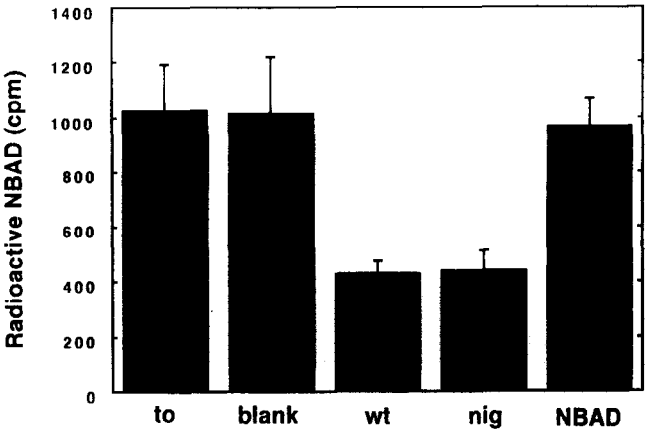

FIGURE 5. *N*-β-Alanyldopamine hydrolase activity in 2 h wild-type and *niger* prepupae of *C. capitata*. [^{14}C]NBAD was used as the substrate. Time zero (t_0) = cpm at the onset of the reaction; blank = reaction mixture incubated without any enzyme; wt = incubation with wild-type extract; nig = incubation with *niger* extract; NBAD = incubation with wild-type extract plus 2 mM unlabeled NBAD. Extracts containing 10 μg of protein from the corresponding strains were used in the assays. Data are the means ± SEM (n = 3).

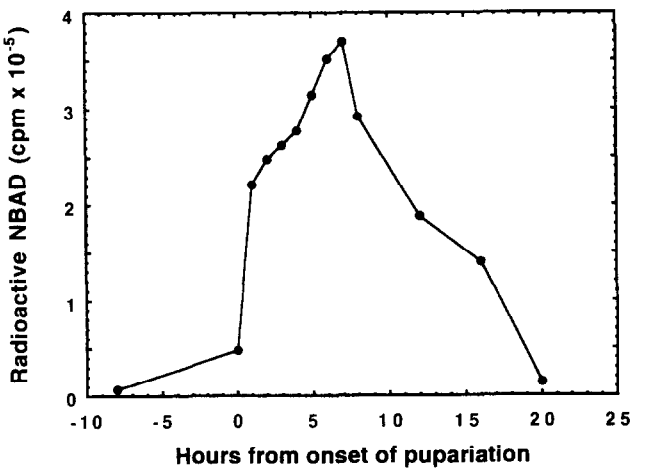

FIGURE 6. Developmental profile of NBAD synthetase activity in wild-type prepupae of *C. capitata*. Ages are expressed in hours before and after the onset of pupariation. Counts per minute (cpm) are per mg protein. Data are the means of two or more determinations.

not significantly different from that in the wild-type strain (Fig. 5).

NBAD synthetase activity during development

Because integumental catecholamines are required only at the immediate postmolt period, it was of interest to investigate whether NBAD synthetase, a key enzyme for sclerotization, was regulated developmentally. At 8 h before the onset of pupariation during the 'jumping larva' stage (Rabossi *et al.*, 1992), enzymatic activity was not detected (Fig. 6). Activity appeared at the onset of pupariation (time 0) and reached the maximum value at 7 h. Activity declined thereafter and was essentially absent at 20 h postpupariation. This result indicates that enzyme expression is restricted to a relatively narrow developmental window that encompasses the period of puparial cuticle sclerotization (Rabossi *et al.*, 1991).

Induction of niger-*wild-type mosaics*

Little information is available about the tissue(s) where NBAD is synthesized (Krueger *et al.*, 1989, 1990). In order to address the tissue localization of NBAD synthesis, we conducted an experiment involving the induction of genetic mosaic puparia. Heterozygous *niger* insects were irradiated during early embryogenesis to generate homozygous somatic clones with a melanic puparial cuticle phenotype. The rationale for this experiment was as follows: if NBAD synthetase is expressed in the epidermis, patches of homozygous *niger* cells would be expected after irradiation, resulting in the appearance of puparial cuticle mosaics. However, if NBAD was synthesized by an internal tissue such as the fat body, cuticular mosaics would not be obtained. In such a case, NBAD would be secreted to the hemolymph,

and therefore, all of the epidermal cells would be expected to incorporate and export to the cuticle similar amounts of the catecholamine. Table 1 shows that puparial mosaics were generated from heterozygous insects obtained from both reciprocal crosses. The areas exhibiting a dark coloration ranged from 5 to 15% of the total puparial cuticle areas. This result indicates that the epidermis is most likely the main tissue expressing NBAD synthetase.

DISCUSSION

We have shown here that the *niger* mutant prepupae of *C. capitata*, unlike the wild type, cannot utilize BALA for NBAD synthesis and subsequent brown coloration of the puparial cuticle. Instead, high concentrations of dopamine accumulate in the hemolymph and cuticle. As has been postulated for other insect species, the excess of dopamine in the mutant is oxidized and polymerized to yield melanin, resulting in the black pigmentation of the puparium (Brunet, 1980; Ujvary *et al.*, 1987; Wright, 1987). In a mechanism that is perhaps similar to what occurs in the *D. melanogaster* mutant *ebony* (Jacobs and Brubaker, 1963; Jacobs, 1966), BALA is not utilized for puparial cuticle sclerotization, but instead is catabolized and eliminated as carbon dioxide. Whereas the *Drosophila* mutant retains more BALA in the internal organs than the wild type (Jacobs, 1968), the BALA content in the internal organs of both the *niger* and wild-type strains of *Ceratitis* is similar. Thus, all of the BALA missing from the melanic puparium of *niger* is catabolized and eliminated as CO_2.

NBAD was the most abundant catecholamine in the wild-type puparium of the medfly (Wappner *et al.*, 1995). NBAD extracted from the cuticle of *niger* was four to five times lower than that extracted from cuticle of the wild-type, whereas dopamine was about 20 times higher in *niger*. In accordance, catecholamine levels in the hemolymph followed a similar pattern with NBAD levels of the wild type being much higher than those of the *niger* strain throughout the recorded developmental period. Dopamine and NADA concentrations were several times higher in the *niger* mutant. This is probably a direct consequence of low NBAD levels, because the excessive dopamine would be either acetylated or not acetylated at all. We recently reported that the *white pupa* mutant of *C. capitata* fails to export catecholamines to the puparium, so they accumulate instead in the hemolymph (Wappner *et al.*, 1995). In contrast, in the *niger* mutant, NBAD is absent from both the cuticle and hemolymph, thus ruling out the possibility of a defect in the distribution of catecholamines because of a failure in the exporting process.

TABLE 1. Numbers of normal and mosaic insects induced by irradiating heterozygous *niger* embryos obtained from both reciprocal crosses

	Normal	Mosaic
♂ wt × ♀ nig	2401	13
♂ nig × ♀ wt	2763	6
♂ wt × ♀ wt	1838	0

We report here, for the first time, *in vitro* determinations of NBAD synthetase and hydrolase activities. These measurements, as well as *in vivo* determinations indicated that NBAD synthesis activity in the wild type was several times higher than that in *niger*, whereas NBAD hydrolase activity was not significantly different. Remarkably, the synthetase activity determined *in vitro* was only four times lower in *niger* than in the wild type, whereas *niger* activity *in vivo* was almost undetectable. A probable explanation for this observation is that the cell-free assay conditions were favorable for NBAD synthetase activity. Although other possibilities might be considered, under such nonphysiological conditions, the defective enzyme of the *niger* mutant might still exhibit partial activity, whereas it might be inactive *in vivo*.

We have shown that NBAD synthetase is regulated developmentally. The temporal pattern of enzymatic activity is in agreement with the developmental changes in hemolymph titers. As expected, the period of maximum synthesis occurs during the first hours of the pupariation process, when the puparium is rapidly tanning.

Irradiation of *nig*/+ heterozygotes leads to the induction of puparial cuticle mosaics. The genetic mechanism underlying the generation of these mosaics has not been studied. Either somatic crossing over, mitotic nondisjunction, or a deletion in the chromosome carrying the wild-type allele could account for the obtained mosaic phenotype. In any case, the success in obtaining isolated patches of cuticle with the *niger* phenotype indicates that NBAD synthetase is expressed in the epidermis. This conclusion is consistent with cuticular melanic patterns observed in virtually all insect orders and might be the result of a regional-specific, epidermal, negative regulation of NBAD synthetase activity or expression. In such a model, NBAD synthetase activity would be restricted to tanned areas of the cuticle and excluded from melanic patches. This possibility is supported by earlier observations of Jacobs (1976), which demonstrated that radiolabeled BALA appeared in tanned areas of the cuticle, but not in the melanic spots when the label was injected into the hemocoele of variegated adult populations of *D. melanogaster*. On the other hand, injected [^{14}C]-dopamine appeared in both dark and light regions of the cuticle.

The origin and function of the hemolymph NBAD pool remain unknown. One possibility, as reported for *Manduca sexta* (Krueger *et al.*, 1989), is that NBAD is synthesized by tissues other than the epidermis. In that case, the hemolymph pool could be a reservoir of NBAD for cuticle sclerotization, which is transported to the cuticle through the epidermis when the tanning process is taking place. An alternative possibility is that the synthesis of NBAD is exclusively epidermal, such as probably occurs in the medfly. In that case, NBAD would be

secreted to the hemolymph through the baso-lateral domain of the epidermal cells.

Dopamine and dopamine-derived substances are found in almost all phylogenetic groups of both invertebrates and chordates, and they function as information-carrying molecules in the central nervous system and also as precursors of pigments in the epidermis. Nevertheless, N-acylated catecholamines are found almost exclusively in the phylum Arthropoda, where they operate as precursors of sclerotizing agents of the cuticle. The evolutionary success of insects relies strongly on the properties of the sclerotized cuticle, which not only protects them from environmental conditions and dehydration, but also provides mechanical support for anchoring the muscles involved in locomotion. Considering that the typical reddish brown insect cuticle is always associated with NBAD-mediated sclerotization (Hopkins and Kramer, 1992), the appearance of epidermal NBAD synthetase could represent a key evolutionary acquisition that favored the radiation of terrestrial arthropods during the paleozoic age. Work is in progress to purify and characterize this developmentally important enzyme.

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